using the Cre-loxP system (PiT1lox/lox; Acan-CreErt2). All pups were injected intraperitoneal with tamoxifen (0.2mg/g body weight) at postnatal day 3 (P3) and harvested at P6, P7 and P10. Histological analyses of long bones were performed by coloration and in situ hybridizations on paraffin section.

Results: Histological analysis of humerus paraffin section at P6 reveals the formation of a hypocellular zone in the center of the growth plate. This hypocellular zone resulted from a massive cell death as shown by TUNEL analysis. The periphery of the neoplastic core was surrounded with smaller, round resting like-chondrocytes. This phenotype resembles to the one observed in mice lacking the hypoxia-inducible transcription factor HIF-1α in cartilage. We are currently investigating the involvement of HIF-1α in the setting up of this phenotype. A similar, but less severe phenotype was observed at P7. At P10 the neoplastic core was not present anymore. However, pronounced disorganization of the proliferative and hypertrophic layers was evident when compared to wild type littermates.

Conclusions: Our findings are in line with our previous observations showing that PiT1-depleted cells are more sensitive to pro-apoptotic signals. Considering the specific chondrocyte microenvironment, PiT1 could represent a key player of the chondrocyte survival, therefore being essential to the growth plate maturation.

284
EFFECTS OF CULTURING TEMPERATURE ON EXTRACELLULAR MATRIX FORMATION AND REDIFFERENTIATION OF EXPANDED HUMAN CHONDROCYTE

A. Ito †,‡, T. Aoyama §, J. Tajino †, M. Nagai †, S. Yamaguchi †, H. Iijima †, X. Zhang †, H. Akiyama ‖, H. Kuroki †. † *Dept. of Motor Function Analysis, Graduate Sch. of Med., Kyoto Univ., Kyoto, Japan;* ‡ *Res. Fellow of Japan Society for the Promotion of Sci., Tokyo, Japan;* § *Dept. of Dev. and Rehabilitation of Motor Function, Graduate Sch. of Med., Kyoto Univ., Kyoto, JAPAN;* ‖ *Dep. of Orthopaedic Surgery, Graduate Sch. of Med., Gifu Univ., Gifu, Japan*

Purpose: Intrinsic capacity of cartilage to repair articular chondral defects is poor. Autologous chondrocyte transplantation technique has been already used for clinical treatment. However, it has been reported that cultured chondrocytes dedifferentiate to fibroblast-like state when cultivated in monolayer condition. While many studies investigating effects of growth factors and scaffolds on chondrocytes have been reported, it is still unknown whether culturing temperature affects on the state of the differentiation and the extracellular matrix (ECM) formation. The purpose of this study was to investigate the effects of the different culturing temperature on the ECM formation and chondrocyte re-differentiation.

Methods: Human primary chondrocytes obtained from femoral head, which was extracted in bipolar hip arthroplasty (62-year-old, female), were expanded in monolayer condition. Thereafter, second passage cells were cultured using pellet culture method for 21 days. Culturing temperature was set at following 3 conditions, 32°C as approximately normal intra-articular temperature, 37°C as inner body temperature and 41°C as threshold temperature of mammal cell survival. The ability of ECM formation was evaluated by measuring wet weight and assessing production of collagen and sulfated glycosaminoglycan (GAG) with histologically and biochemically. Furthermore, the synthesized ECM ultrastructure was observed using scanning electron microscope (SEM). To assess the mechanical property of the synthesized ECM, unconfined compression test was applied for measuring a peak stress. The differentiation state of the chondrocyte was evaluated by gene expression analysis including type II collagen (COL2), type I collagen (COL1), aggrecan (ACAN) and cartilage oligomeric matrix protein (COMP), and by immunohistochemical staining of COL2 and COL1.

Results: The wet weight during 3-21 days was increased with time at 32°C and 37°C, but unchanged at 41°C, and that of 41°C was significantly lighter than that of 32°C and 37°C until culturing day 21. There were no significant differences between 32°C and 37°C. In histological evaluation with picrosirius red and safranin-O staining, collagen and GAG production were low at 41°C compared with 32°C and 37°C at day 7, 14 and 21. Safranin-O staining of 37°C appeared to be higher than 32°C. In biochemical evaluation with DMMB assay, GAG content at 41°C was also significantly lower than 32°C and 37°C at day 14 and 21, and that of 37°C was higher than 32°C at day 21. In SEM observation at day 21, dense and layered collagen fiber formations were observed in the peripheral region at 32°C and 37°C, whereas there are no collagen formations were observed at 41°C. The peak stress was higher at 32°C than that of 37°C and 41°C between day 7 and 14. The mRNA expressions of all examined genes were down-regulated at 41°C compared with 32°C and 37°C at day 3 and 7. There was no significant difference on COL2 mRNA expression between 32°C and 37°C, whereas the expression was up-regulated with time. COL1 mRNA was up-regulated at 32°C compared with 37°C at day 7. While ACAN mRNA expression was up-regulated at 32°C compared with 37°C at day 3, there was no significant difference between 32°C and 37°C at day 7. COMP mRNA expression was up-regulated at 37°C compared with 32°C at day 3 and 7. Immunohistochemical staining of COL2 could not detect even at day 21 at all temperature. On the other hand, that of COL1 was detected from day 7 at 32°C and 37°C, and was increased with time.

Conclusions: Our results using human chondrocyte showed that cartilage ECM formation in pellet culture method was dramatically inhibited at 41°C. This result was consistent with previous report which showed that the collagen cannot fold into a triple-helix conformation at a temperature of approximately 40°C. Therefore, long term exposure of mild heat stimulus (41°C) could cause adverse effects on ECM formation, although intermitted mild heat stimulus could be enhance collagen production. Interestingly, 32°C, which is comparatively lower temperature condition, had the ability of ECM formation equivalent to inner body temperature (37°C). Our results also indicated that chondrogenic gene expression and GAG production were enhanced at both 32°C and 37°C with time, and based on GAG production, 37°C might enhance redifferentiation compared with 32°C.

285
BCP CRYSTALS INDUCE HYPERTROPHIC DIFFERENTIATION OF CHONDROCYTES BY ACTIVATING CANONICAL WNT SIGNALING

T. Kräft †, L. Godmann †, J. Hubert ‡, Y. Nitschke §, T. Hawellek ‡, F. Liote ‖, T. Pap †, J. Bertrand †. † *Inst. for Experimental Musculoskeletal Med., Münster, Germany;* ‡ *Klinik für Orthopädie und orthopädische Rheumatologie, Hamburg, Germany;* § *Dept. of Pedriatricts, Univ. Hosp. Münster, Münster, Germany;* ‖ *Univ. Paris Diderot, Sorbonne Paris Cité, Inserm, UMR 606, Paris, France*

Purpose: Calcification of cartilage is a common finding during osteoarthritis (OA) and is directly linked to the severity of cartilage degradation. We have found in a previous study that basic calcium phosphate (BCP) crystal calcification is present in murine and human OA cartilage. The observed cartilage changes resemble aspects of endochondral ossification. In this study we aim to investigate the effect of BCP crystals on articular cartilage matrix changes.

Methods: The tip-toe walking (ttw/ttw) mouse that carries a mutation in the enpp1 gene encoding for NPP1 was used as a natural model of OA. Using von Kossa staining of knee joint sections we assessed the calcification of articular cartilage and the severity of OA using the Mankin-Score over a time course from 8 to 22 weeks and compared the results to DMM induced OA. We analysed the influence of octacalcium phosphte crystals on chondrocyte phenotype using quantitative RT-PCR for the marker genes MMP13, PCNA and aggrecan. We compared these findings with data from ttw/ttw micromass cultures. The influence of BCP crystals on matrix composition in vitro was investigated in micro mass cultures with alcian blue and alizarin red staining. Using Western Blot for β- catenin and pCamKII we investigated the activation of WNT signalling. Using FURA-2 measurements we investigated the effects of BCP on Ca2+ mobilization in chondrocytes.

Results: We found a loss of proteoglycans and increase in the Mankin Score in ttw/ttw mice already at an age of 8 weeks, whereas no changes of cartilage matrix were detectable in wild type mice. Early matrix remodelling in ttw/ttw cartilage was followed by an increased calcification starting at the border to the subchondral bone, with a break down of the straight border of the tidemark. The same effect on the tidemark was observed in wild type mice after DMM induced OA. Investigating the effects of BCP crystals on the chondrocyte phenotype we found an increased expression of the hypertrophy marker MMP13